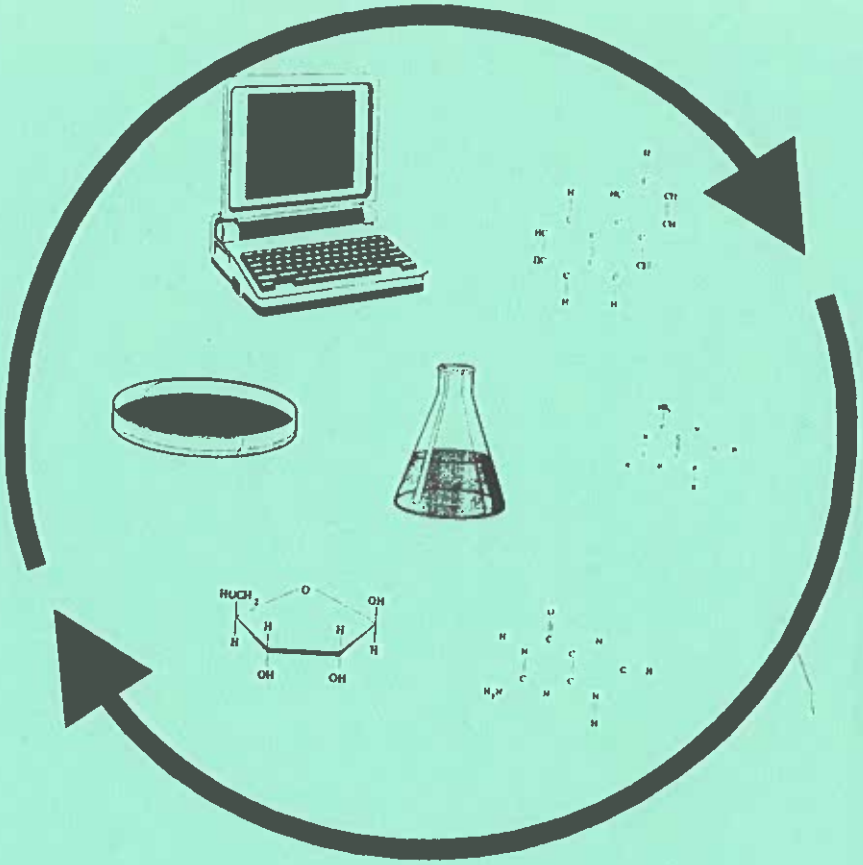
31ST PLANT DEVELOPMENT WORKSHOP



PROGRAM and ABSTRACTS

Department of Plant Sciences
University of Western Ontario

November 16, 1996

31st PLANT DEVELOPMENT WORKSHOP
November 16, 1996
University of Western Ontario
Western Science Center (Rm. 055)

8:30 a.m **_Registration and Coffee, WSC_ 055**

9:15 a.m. Welcome - D.B. Walden, DPS, U.W.O.

MORNING STARTER:

9:20 a.m. **van Huystee, R.B.**
Department of Plant Sciences, University of Western Ontario, London, Ontario.
The structure and function of a cell wall enzyme.

SESSIONAL CHAIR: R. B. van Huystee

10:00 a.m. **Cholewa, E. and C.A. Peterson.**
Department of Biology, University of Waterloo, Waterloo, Ontario.
Elucidation of the pathway of solutes transport in onion roots.

10:15 a.m. **Davidson, A. and W. Newcomb.**
Department of Biology, Queen's University, Kingston, Ontario.
Organization of actin filaments in pea root nodule cells.

10:30 a.m. **Uetake, Y. and R.L. Peterson.**
Department of Botany, University of Guelph, Guelph, Ontario.
Actin filament arrays in symbiotic orchid protocorms.

10:45 a.m. *COFFEE BREAK*

11:00 a.m. **Morgan, R.M., Ivanov, A.G., Maxwell, D.P. and N.P.A. Huner.**
Department of Plant Sciences, University of Western Ontario, London, Ontario.
Characterization of the light harvesting complex I (LHC I) in the Antarctic green alga, *Chlamydomonas subcaudata*.

11:15 a.m. **Ma, F., Kieft, H.[1] and A.A.M. van Lammeren[1].**
Department of Biology, University of Waterloo, Waterloo, Ontario, [1]Department of Plant Cytology and Morphology, Wageningen Agricultural University, Arboretumlaan, The Netherlands.
Ultrastructure of early endosperm development in *Brassica napus* L.

11:30 a.m. **Lynch, J.M. and A.M. Zobel.**
Department of Chemistry, Trent University, Peterborough, Ontario.
Short and long-term influence of UV-A radiation on *Brassica oleracea*.

11:45 a.m. *LUNCH*

1:15 p.m. POSTERS (Biological and Geological Room 217)

2:15 p.m. Introduction - D.B. Walden

AFTERNOON STARTER:

2:20 p.m. **Cheng, P.-C.**
Advanced Microscopy and Imaging Laboratory, Department of Electrical and Computer Engineering, State University of New York at Buffalo, Buffalo, NY.
Three-dimensional imaging technology for botanical research.

SESSIONAL CHAIR: N.P.A. Huner, DPS, U.W.O.

3:00 p.m. **Lemon, G.D. and U. Posluszny.**
Department of Botany, University of Guelph, Guelph.
Shoot morphology and organogenesis of the aquatic floating fern *Salvinia molesta* examined with the aid of laser scanning confocal microscopy.

3:15 p.m. **Soros, C.L. and N.G. Dengler.**
Department of Botany, University of Toronto, Toronto, Ontario.
Quantitative leaf anatomy of C_3 and C_4 Cyperaceae and comparisons with the Poaceae.

3:30 p.m. **Stevens, K.J.[1], Peterson, R.L.[1] and G.R. Stephenson[2].**
[1]Department of Botany, [2]Department of Environmental Biology, University of Guelph, Guelph, Ontario.
Morphological and anatomical responses of *Lythrum salicaria* L. (purple loosestrife) to an imposed water gradient.

3:45 p.m. **Evans, R.C. and T.A. Dickinson.**
Department of Botany, University of Toronto, Toronto, Ontario and Centre for Biodiversity and Conservation Biology, Royal Ontario Museum, Toronto, Ontario.
A microscopic study of early ovary and ovule development in the Rosaceae - where does the variation come from?

4:00 p.m. *COFFEE*

4:30 p.m. *WRAP-UP SESSION, ANNOUNCEMENTS:* D.B. Walden

POSTERS

Biological and Sciences Bldg., Room 217

9:15 a.m. - 5:00 p.m.

1:15-2:15 p.m. AUTHOR(S) PRESENT

1 **Scholey, C.A., Waite, J.L. and C.A. Peterson.**
Department of Biology, University of Waterloo, Waterloo, Ontario.
Onion exodermal short cells: their role in symplastic transfer.

2 **Bates, K.J., Conn, K. and G. Lazarovits.**
Agriculture & Agri-Food Canada, Pest Management Research Centre, 1391 Sandford St., London, ON
Growth-promotion of potato (*Solanum tuberosum*) by a nonfluorescent *Pseudomonas* sp. (PsJN).

3 **Niiquaye, E.Q. and S.L. Jackson.**
Department of Plant Sciences, University of Western Ontario, London, Ontario.
Exogenous calcium concentration can influence growth and morphogenesis in the hyphae of *Basidiobolus ranarum*.

4 **Schelkle, M., Kronick, M., Farquhar, M. and R.L. Peterson.**
Department of Botany, University of Guelph, Guelph, Ontario.
The use of laser scanning confocal microscopy to characterize ectomycorrhizas of *Pinus strobus* L. and to localize associated bacteria.

5 **MacGregor, T.[1], Bhat, R.[2] Schmitthenner, F.[2], Bhattacharyya, M.[3] and M. Gijzen[4].**
[1]Department of Plant Sciences, The University of Western Ontario, London, Ontario, [2]Ohio State University, Wooster OH, [3]Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK, [4]Agriculture and Agri-Food Canada, 1391 Sandford St., London, Ontario.
Genetic mapping of molecular markers linked to the *Avr1a* Avirulence gene in the soybean pathogen *Phytophthora sojae*.

6 **Reid, D.A. and J.N.A. Lott.**
Department of Biology, McMaster University, Hamilton, Ontario.
Energy dispersive x-ray analysis of white spruce seeds and somatic embryos.

7 **Clarke, P.A., Zobel, A.M. and J.M. Lynch.**
Department of Chemistry, Trent University, Peterborough, Ontario.
The effects of UV-A irradiation on the production of phenolic compounds in seedlings of *Acer saccharum* and *Acer platanoides*.

8 **Pocock, T., Savitch, L. and N.P.A. Huner.**
Department of Plant Sciences, University of Western Ontario, London, Ontario.
The UV-A induced resistance to UV-B stress in relation to photosynthetic carbon metabolism in *Brassica napus* cv Topas.

9 **Sud, R.M. and N.G. Dengler.**
Department of Botany, University of Toronto, Toronto, Ontario.
Distribution of chlorophyll and photosynthetic enzymes in the variegated C4 grass, *Stenotaphrum secundatum*.

10 **Switzer, T., Shoneberger, S., Lynch, J. and A. Zobel.**
Department of Chemistry, Trent University, Peterborough, Ontario.
Influence of ions on secondary metabolites in plant shoots.

11 **Ockenden, I.[1], Falk, D.E.[2] and J.N.A. Lott[1].**
[1]Department of Biology, McMaster University, Hamilton, Ontario, [2]Crop Science Department, University of Guelph, Guelph, Ontario.
Phytate in stored barley grains and bean seeds.

12 **Nicholson, M.L. and D.E. Laudenbach.**
Department of Plant Sciences, University of Western Ontario, London, Ontario.
Genes encoded on a cyanobacterial plasmid are transcriptionally regulated by sulfur-availability and CysR.

13 **Kohalmi, S.E.[1], Ritchie, S.[2], Nowak, J.[2] and W.L. Crosby[2].**
[1]Department of Plant Sciences, University of Western Ontario, London, Ontario, [2]NRCC, Plant Biotechnology Institute, Saskatoon, SK.
The MADS-box protein AGL2 is able to interact with floral regulators of *Arabidopsis thaliana*.

14 **Denison, S.J., Smith, R.J. and D.B. Walden.**
Molecular Genetics Unit, Department of Plant Sciences, University of Western Ontario, London, ON
The use of immuno-gold cytochemistry to determine the sub-cellular localization of the 18-kDa heat-shock proteins in maize radicles.

15 **Yang, Z., Greyson, R.I., Bouchard, R.A. and D.B. Walden.**
Molecular Genetics Unit, Department of Plant Sciences, University of Western Ontario, London, ON
Antisense RNA *in situ* hybridization reveals the localization of mRNAs of 18 kDa heat-shock protein genes in metal-ion insulted maize radicles.

16 **Lige, B. and R.B. van Huystee.**
Department of Plant Sciences, University of Western Ontario, London, Ontario.
Expression of peanut peroxidase in tobacco.

17 **Sun, Y. and R.B. van Huystee.**
Department of Plant Sciences, University of Western Ontario, London, Ontario.
Glycan analysis of cationic peanut peroxidase.

18 **Maillet, D.S. and D.B. Walden.**
Molecular Genetics Unit, Department of Plant Sciences, University of Western Ontario, London, ON
Genome organization in *Zea mays* L.

9:20 a.m. *Morning Starter*

THE STRUCTURE AND FUNCTION OF A CELL WALL ENZYME

R. B. van Huystee.
Department of Plant Sciences, University of Western Ontario, London, Ontario N6A 5B7

A peroxidase, isolated from a spent culture, has been identified as a cell wall enzyme by immunogold labelling. Its function as a growth-retardant was first shown by arrest of hypo-cotyl growth when grown on agar, containing peroxidase. Then, assays with extensin and ferulic acid as substrate suggested intermolecular linkages to be formed which may arrest cell-wall extension. Arrest of hypocotyl growth, on meta-fluoro-tyrosine containing agar caused a decline of total proteins but an increase of the cell wall peroxidase, measured by ELISA. This increase was not due to synthesis. Conversely, pulse-chase experiments showed that this peroxidase has a longer half life in stressed cells. What may be the reason for this ?

Peroxidase has several prosthetic groupings. Is there any function for such groupings ? Since peroxidase is a oxido-reductase enzyme, heme is essential for the reaction site. Calcium is needed for its enzyme activity but its precise role was unknown. Using NMR analysis of the active site, calcium was shown as essential for the conformation of the active site. Protein crystalization and X-ray diffraction confirmed the relationship of calcium to the heme active site. It is now reasonable to assume that in the absence of calcium the protein chain is relaxed and under in vivo conditions open to attack by protease(s). Calcium may be a stabilizing factor !

In addition, three N-linked glycans sites have been found on the cDNA and the protein product of that gene. Protein cleavage and amino acid sequence have established their relationship. Three putatively different "complex" glycans have been identified for each of these three sites. The question remains why there should be 3, and such variation in the glycans on each site ? Two forms of this enzyme differing only in the glycan length have been separated on Con-A affinity. Preliminary data point to ß-galactosidase, co-secreted in the medium. It may cause the variation. The enzyme activity of both forms does not differ. So, the terminal loss of the galactose molecules does not affect the activity. Conversely, when the peroxidase is treated with PNGase F the enzyme activity is lost but structural variations, observed by MCD, in the protein and the heme pattern occur. This is not due to the loss of one or more entire glycan groups since this endoglyco-peptidase is known to be unable to attack glycans with proximal fucose linkages.

We are pursuing glycan analysis of the cell wall peroxidase to determine their role in enzyme stability. Attempts of site-directed mutagenesis are underway. In addition, glycosidase found in the inter-cellular spaces may determine the half-life of the enzyme ! Studies of glycosidase effects on the stability of the cell wall peroxidase are also in progress.

In complex enzymes it may not always be the synthesis of the enzyme that is important but it may also be the prosthetic groups that may influence the half-life of the enzyme.

Morning Sessional Chair: R. B. van Huystee

10:00 a.m.

Elucidation of the pathway of solutes transport in onion roots.

Ewa Cholewa and Carol A. Peterson, Department of Biology, University of Waterloo, ON N2L 3G1.

Water and ions are taken up by onion roots and transported through the xylem to the leaves. The anatomy of onion root is well described (Perumalla and Peterson, Can. J. Bot. 64:1873, 1996). It was established that in onion roots, Casparian bands are present in the exo- as well as in the endodermis. They block the radial apoplastic movement of water and ions from the soil solution to the stele in mature roots, as it was demonstrated for the sulfate ion (Peterson, J. Exp. Bot. 38:2068,1987). It is proposed, therefore, that a combination of pathways for ion transport exists in young onion roots - apoplastic around the endodermis in the root tip area, and symplastic through the plasmodesmatal connections between the endodermis and xylem parenchyma. Recent results obtained in our lab revealed that there is no apoplastic pathway in young, growing onion roots. Six to nine day-old intact onion plants (sprouted from bulbs) were treated with the fluorescent apoplastic dye PTS, and allowed to transpire for various lengths of time. No PTS was detected in the aqueous extracts of the leaves, indicating that PTS was not transported with the mass flow of water. This may mean that the transport of all ions occurs through the plasmalemma and cytosol. However, PTS is larger than ion. Therefore further studies will be performed with radio-tracers to verify this finding. Observation of whole roots treated with PTS confirmed that the exodermis is blocking apoplastic movement in the mature region of the root. PTS was present in the apoplast of the cortical parenchyma in the root regions with immature exodermis and absent in older regions of the root. This observation was correlated to the presence or absence of the Casparian bands in the exodermis. Further studies with different plant species are required to validate the technique of the use of PTS as a rapid indicator of the presence of exodermis in roots.

10:15 a.m.

Organization of Actin Filaments in Pea Root Nodule Cells

Queen's University, Department of Biology
Authors - Andrea Davidson and William Newcomb

Root nodules are small growths on the roots of leguminous plants. In pea, *Pisum sativum*, these structures are the result of root tissue proliferation stimulated by the nitrogen fixing bacterium *Rhizobium leguminosarum*. The rhizobia infect the developing root nodule via infection threads that originate in root hairs. These tube-like structures, lined by host cell wall and host plasma membrane, grow into the cortical tissue of the root. Bacteria are released from unwalled regions of the infection thread into the centrally located cortical cells of the nodule. The bacteria replicate, enlarge, and begin to occupy a significant portion of the cell cytosol. The infected cell also increases in size, and mitochondria are relocated to the peripheral regions of the cell. Given the nature of the infection process and the alteration that occurs in the infected plant host cell one would expect that the plant cytoskeleton would also be disrupted.

Fluorescence microscopy and laser confocal microscopy were utilized to determine how actin filaments are reorganized in infected root cells compared to uninfected cells. This relationship will be observed across a developmental gradient in the developing pea nodule.

10:30 a.m.

Y. Uetake and R.L. Peterson. Department of Botany, University of Guelph, Guelph, Ontario, N1G 2W1 Canada

Actin filament arrays in symbiotic orchid protocorms.

Actin filaments were observed in cultured symbiotic protocorms of *Spiranthes sinensis* formed as a result of the colonizaiton of imbibed seeds by the symbiotic fungus, *Ceratobasidium cornigerum* (AG-C). Protocorms were either fixed with 4% paraformaldehyde , sectioned by hand in buffer, and the sections stained with rhodamine-phalloidin (rh/ph), or fresh materials were directly sectioned and stained in rh/ph. Sections were observed by laser scanning confocal microscopy. In uncolonized cells, actin filaments forming thick bundles and fine filaments were mostly located in the cell cortex and were also observed around nuclei. In colonized cells, in which the cycle of formation and degeneration of a hyphal coil (peloton) occurred, the cortical bundles of actin filaments radiated from the early peloton or degenerated hyphal mass. Actin bundles were also observed along hyphae of the peloton which occupied most of the host cell at the mature stage of colonization. Actin filament arrays observed in this study showed less physical association with fungal hyphae compared to microtubule arrays which are in close association with hyphal structures in similar symbiotic orchid protocorms.

10:45 a.m COFFEE

11:00 a.m.

CHARACTERIZATION OF THE LIGHT HARVESTING COMPLEX I (LHC I) IN THE ANTARCTIC GREEN ALGA, *CHLAMYDOMONAS SUBCAUDATA* **Morgan RM, Ivanov AG, Maxwell DP, Huner NPA** Dept. of Plant Sciences, University of Western Ontario, London, ON

Growth kinetic analysis confirmed that the Antarctic green alga, *Chlamydomonas subcaudata*, is a psychrophile, with an optimum growth temperature of 10°C. When cultures of *C. subcaudata* were grown at the control temperature (8°C) and varying irradiances (20, 150 or 300 μE), it was observed that the photosynthetic response is different in *C. subcaudata* than that of mesophilic algal species grown under similar conditions. For example, *Chlorella vulgaris* and *Dunaliella salina* photosynthetically adjust to low temperature or high light by down-regulating the light harvesting complex II (LHC II) polypeptides and decreasing chlorophyll (Chl) per cell. Thus, it was concluded that *C. subcaudata* may utilize different acclimatory strategies than mesophilic algae.

Analysis of Chl *a* fluorescence at 77 K in *C. subcaudata* indicated that the fluorescence emission spectrum lacked a typical maximum at 710-720 nm, representing fluorescence associated with photosystem I(PS I)-related polypeptides. However, SDS-PAGE and subsequent immunoblotting of the thylakoid polypeptides confirmed that the psychrophile does possess the PS I holocomplex. Furthermore, characterization of the Chl-binding complexes in *C. subcaudata* versus a mesophile, *C. reinhardtii*, indicated that although the abundance of LHC II polypeptides is comparable between the two species grown under varying conditions, LHC I appears to be greatly reduced in the psychrophile. This characteristic is a consequence of overall lower levels of PS I in *C. subcaudata*, but in addition appears to involve the reduction or lack of specific LHC I polypeptides. It is postulated that the apparent contradiction between the results of the 77 K fluorescence, suggesting a lack of PS I, and immunoblotting, which confirmed the presence of PS I, may be a consequence of a different type of LHC I organization in the psychrophile in comparison with *C. reinhardtii*. This could result in a shift in the 77 K fluorescence maximum of PS I to lower wavelengths in *C. subcaudata*.

11:15 a.m.

Ultrastructure of early endosperm development in *Brassica napus* L.

Fengshan Ma, [1] Henk Kieft and [1] André A.M. van Lammeren
[1]Department of Biology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada, and [2]Department of Plant Cytology and Morphology, Wageningen Agricultural University, Arboretumlaan 4, NL-6703 BD Wageningen, The Netherlands

Cytology and early alveolation of the nuclear endosperm of *Brassica napus* L. were investigated by light and electron microscopy. Morphological heterogeneity existed in the micropylar, lateral and chalazal regions of the nuclear endosperm. The lateral region, which had a thin layer of endosperm, differed from the micropylar and chalazal regions where accumulation of cytoplasm and nuclei was conspicuous probably caused by the shapes of these regions. Also extensive wall ingrowths were formed on the central cell wall in chalazal and micropylar zones. They probably mediate nutrient uptake and translocation from the integuments to the endosperm. The chalazal endosperm seemed most active in metabolism, as indicated by the position and high numbers of organelles in the cytoplasm.

In the lateral regions of the central cell, anticlinal walls were the result of cell plate formation between regularly spaced nuclei of the coenocyte, with the involvement of microtubules. Rough endoplasmic reticulum was observed in close contact with the developing cell walls and might play a role in holding wall pieces during wall extension and in transferring wall materials during wall maturation. Although protrusions of the central cell wall might be potential fusion sites of anticlinal walls, they were not generated at such sites. Cellularization of the chalazal endosperm proceeded without alveolation since nuclei were not organized in a monolayer.

11:30 a.m.

Short and Long-Term Influence of UV-A Radiation on *Brassica oleracea*

J.M. Lynch*, and A.M. Zobel
Department of Chemistry, Trent University, Peterborough, ON, Canada. K9J 7B8

Phenolic compounds of plants may play several important ecological roles including defence and protection from environmental stress, particularly UV radiation. The purpose of this experiment was to investigate the short-term and long-term influence of UV radiation on ornamental red cabbage. Fifteen leaves were removed from one *Brassica oleracea* var. *acephala* plant: 3 leaves served as the start-point; 3 leaves were exposed to 366 nm radiation for 2 days; 3 leaves were left in darkness for 2 days; 3 leaves were exposed to 366 nm radiation for 7 days; and, 3 leaves were left in darkness for 7 days. Following the treatment, the surface UV absorbing phenolic compounds were removed along with the wax using almost boiling water, 96^{0}C (Zobel and Brown, 1988), and the interior contents were extracted using a H_2O:EtOH:HCl (79:20:1) mixture (Van Sumere *et al.*, 1985). The absorption spectra were recorded at 325 nm by a UV / visible spectrophotometer. The results indicate that UV radiation changed the concentrations of these compounds and enhanced extrusion after 48 hours of continuous radiation. After 7 days concentrations of the surface and interior phenolics were similar to the control leaves thus, the red cabbage would seem to have adapted to the stress of 366 nm UV radiation. Several repetitions have rendered similar findings.

2:20 p.m. *Afternoon Starter:*

THREE-DIMENSIONAL IMAGING TECHNOLOGY FOR BOTANICAL RESEARCH

Ping-chin Cheng
Advanced Microscopy and Imaging Laboratory, Department of Electrical and Computer Engineering, State University of New York at Buffalo, Buffalo, NY 14260

Three new technologies-confocal microscopy, two-photon fluorescent microscopy and X-ray microtomography - are currently available for the study of botanical structures in three dimensions. This presentation will provide an introduction to these technologies and address their potential applications in plant biology.

Confocal fluorescent microscopy has become a standard technique in the study of three-dimensional organization of biological structures. The capability of obtaining optical sections in multiple spectral channels has made the technology an indispensable tool for understanding cellular structures and functions by using various fluorescent tags. The use of confocal microscopy in botanical studies, however, frequently suffers from high autofluorescent, scattering and absorbance of the specimens.

Two-photon fluorescent microscopy has recently become a useful tool in the study of biological specimens in 3D. The technique achieves optical sectioning even without the use of conventional confocal optics. The wavelength of the excitation light is generally in the near IR spectrum (e.g. 800 nm); up-converted visible fluorescence is detected to form the image. Because of the low absorbance of IR light in biological specimens, this technique enables deep probing of a specimen and is of particular interest to plant biologists. In addition, photo-bleaching of fluorophores is restricted near the vicinity of the focal spot and autofluorescent of the specimen is greatly suppressed in two-photon fluorescent microscopy. Current results demonstrate that optical sections can be obtained from a depth more than 300 um from the surface of the specimen.

In addition to the optical methods described above, recent development in X-ray microtomography has been applied to the study of structural organization in optically opaque materials. The technique can be use for visualizating woody specimen or bulky plant samples. The basic principle of X-ray microtomography and examples of botanical applications will be discussed. Multi-dimensional image process and analyses specially developed for handling data obtained from single and two-photon confocal microscopy and X-ray microtomography will be presented.

Afternoon Sessional Chair: N.P.A. Huner

3:00 p.m.

LEMON, GORDON D.* and USHER POSLUSZNY. Department of Botany, University of Guelph, Guelph, Ontario, N1G 2W1 - Shoot morphology and organogenesis of the aquatic floating fern *Salvinia molesta*: examined with the aid of laser scanning confocal microscopy

This study examines the unique apical meristem of the aquatic floating fern *Salvinia molesta*, using epi-illumination microscopy, resin sectioning, scanning electron microscopy (SEM) and a new and potentially very useful technique, laser scanning confocal microscopy (LSCM). The use of LSCM provides a superior method for the analysis of three dimensional aspects of apical meristem development. There are four structures which form at a node of *S. molesta*; two floating leaves, one submerged leaf, and a lateral bud. The first primordium to initiate is that of the of the distal floating leaf, followed by the lateral bud, the proximal floating leaf, and finally the submerged leaf. The primordia develop in the same order as their inception, which contradicts a previous study that claimed that the primordia developed in the reverse order of their initiation. As well, this study reveals for the first time that *S. molesta* produces serial lateral buds, at times as many as five in a mature node. These serial lateral buds appear to be unique among pteridophytes and are certainly rare among seed plants. Understanding the developmental pattern in *S. molesta* has helped clarify the incredible growth potential of this aggressive water weed.

3:15 p.m.

SOROS, CONNIE, L.* & NANCY G. DENGLER. Department of Botany, University of Toronto, Toronto, Ontario. M5S 1A1, Canada. - Quantitative leaf anatomy of C_3 and C_4 Cyperaceae and comparisons with the Poaceae.

In C_3 plants, the major type of photosynthetic chloroplast containing cell is the mesophyll. In contrast, a typical C_4 leaf has two distinct chloroplast containing cell types, the PCA and the PCR (Kranz) which must function together in order for the PCA cycle to operate. We examined species of C_3 and C_4 anatomical types of Cyperaceae and Poaceae to evaluate generalizations about C_4 anatomy based on earlier observations made on the Poaceae. In C_4 Cyperaceae, different anatomical types have one or two cell layers intervening between PCA and PCR tissue, thus breaking the "maximum cells distant count" rule for C_4 anatomy. These "extra" layers may contribute to the diffusional barrier, and with one or more suberized layers, add support to the hypothesis that certain anatomical features reduce apoplastic leakage of CO_2 from PCR to internal cellular space. Newly expanded leaves from replicate plants of each species were embedded in resin for analysis with the light microscope. Quantitative measurements and qualitative descriptions of anatomical characters between adjacent cell types were assessed. Our observations revealed that the absolute distances between PCA and PCR tissues are approximately equivalent in the Poaceae and different anatomical types of the Cyperaceae regardless of intervening tissue layers. This would indicate that although extra layers may be present to reduce apoplastic leakage of CO_2, these layers do not impede the efficient metabolite transfer between the layers required for C_4 photosynthesis. We also found that although the Cyperaceae have a consistently smaller percentage of PCR tissue, percent chloroplast number within the PCR tissue is conserved between the Cyperaceae and the Poaceae. Despite differences in developmental pattern, the essential features of C_4 anatomy are achieved by leaf maturity, indicating that functional constraints are imposed on differing evolutionary origins of the C_4 syndrome.

3:30 p.m.

K.J. Stevens[1], R.L. Peterson[1] and G.R. Stephenson[2]. [1]Department of Botany, [2]Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1
Morphological and anatomical responses of Lythrum salicaria L. (purple loosestrife) to an imposed water gradient.

Morphological and anatomical responses of Lythrum salicaria L. to an imposed water gradient were assessed under greenhouse conditions. Nine week old seedlings were exposed to three levels of water availability for an eight week period. Fresh and dry mass of the shoot, root and entire plant, shoot fresh:dry mass ratios, root:shoot fresh mass ratios and root:shoot dry mass ratios were not significantly affected by water availability. Total fresh:dry mass ratios were significantly lower in the dry treatment compared with the wet, while root fresh:dry mass ratios were significantly greater in the intermediate and wet treatments. Total stem diameter and porosity was generally higher in submersed portions of flooded stems and roots, while tissue density was generally lower under these conditions. Stem diameter excluding the phellem did not differ among treatments. The total root diameter to diameter excluding phellem showed significant increases with increasing water availability. Adventitious and lateral roots in primary growth from all plants possessed an endodermis with Casparian bands and subsequently suberin lamellae and a modified, uniseriate hypodermis. Submerged stems and roots in secondary growth possessed a multi-layered, lacunate polyderm, while non-submerged stems and roots had a compact, multi-layered polyderm. Fluorescent properties of walls of some cells in the root and stem polyderm indicate that these cells share features with endodermal cells.

3:45 p.m.

Evans, Rodger, C.* and Timothy A. Dickinson. Department of Botany, University of Toronto, Toronto, ON, M5S 3B2 and Centre for Biodiversity and Conservation Biology, Royal Ontario Museum, Toronto, ON M5S 2C6. A microscopic study of early ovary and ovule development in the Rosaceae - Where does the variation come from?

The Rosaceae are a large group of flowering plants which exhibit a wide level of variation in ovary and ovule morphology. Gynoecium morphology in the Rosaceae varies from apocarpy through to complete syncarpy. While ovary/hypanthium associations can vary from free through to complete adnation. Rosaceous ovules are formed from axile placentation, are anatropous, and vary in position from pendulous, to lateral, to basal. Ovules also vary in number as well as in position to each other within the locule. As part of a larger phylogentic investigation of the Rosaceae, and in particular the evolution of subfamily Maloideae, we have chosen a subset of taxa with which to investigate early floral development. The taxa we have chosen include members of subfamilies Prunoideae, Spiraeoideae, and Maloideae. Preliminary results indicate that variation in ovary morphology is determined at the time of gynoecial initiation. In prunoids and some spiraeoids the gynoecia are initiated as distinct primordia. This differs from other spiraeoids, and all maloids, in which a ring primordium is the first indication of gynoecial development. Further differentiation into individual primordia appears to be through differential development of the distal portion of the ring primordium. This differential development may also play a role in the degree of ovary connation. The proximity of the dorsal portion of gynoecial primordia to the developing hypanthium early in floral development, and subsequent hypanthium growth, appears to determine perigny and epigyny. In all taxa studied ovule initiation is on the ventral margin of the ovary. Ovules typically develop collaterally, but superposition of ovules occurs in some taxa. Associated with each ovule is an obturator that may develop from the funiculus or the ovary margin. The ultimate goal of this early development study is to place pathways of early floral development into a phylogenetic context in order to produce hypotheses concerning the evolution of the Maloideae.

POSTERS

Biological and Sciences Bldg., Room 217

9:15 a.m. - 5:00 p.m.

1:15-2:15 p.m. AUTHOR(S) PRESENT

1 ONION EXODERMAL SHORT CELLS: THEIR ROLE IN SYMPLASTIC TRANSFER

Scholey, C.A., Waite, J.L. and Peterson, C.A., Dept. of Biology, University of Waterloo, Canada, N2L 3G1

The onion root possesses a dimorphic exodermis comprised of long and short (or passage) cells. In the long cells, Casparian bands and suberin lamellae develop nearly concurrently whereas in the short cells, suberin lamella development is delayed or absent. Because the Casparian bands prevent the diffusion of ions, those taken up by a root zone in which the exodermis is mature enter the symplast (cytoplasm) of the epidermal or short exodermal cells. Further inward movement would occur via plasmodesmata. We tested for these symplastic connections using fluorescein applied in three ways; i) to the epidermis only, ii) to both the epidermis and cortex, or iii) to the cortex only (by injection into the intercellular air spaces). Results of these studies indicated that the long cells of the exodermis were connected only to the internal cortical cells, and not to either the epidermal or short exodermal cells. The short cells, on the other hand, were connected to both the epidermis and underlying cortex and would provide an entry port for ions.

2 **Growth-promotion of Potato (*Solanum tuberosum*) by a nonfluorescent *Pseudomonas sp.* (PsJN)**

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A nonfluorescent *Pseudomonas sp.* (PsJN) bacterium was found to enhance the growth and development of potato plants under tissue culture conditions. In order to examine the genetic basis for plant growth-promotion, nongrowth-promoting mutants of PsJN were generated by Tn_5 transposon mutagenesis. Over 2000 mutants were originally screened on potato nodes for the loss of growth-promotion. A nongrowth-promoting mutant (H41) was selected and a genomic library was generated. DNA probes derived from the mutant genomic DNA library were used to screen a PsJN genomic library for homologous DNA sequences. A restriction map was generated for nine PsJN and five H41 genomic DNA clones. Shotgun cloning of various DNA fragments was used to generate over 100 specific clones (pKK). Fifteen clones representing PsJN fragments adjacent to the Tn_5 transposon insertion point were selected for complementation of H41. After 6 weeks, growth-promotion was restored in the nongrowth-promoting Tn_5 mutant by five clones, each consisting of the same 7 kb PsJN genomic DNA fragment. Therefore, we have identified a region of PsJN DNA capable of inducing growth-promotion in potato nodal plants.

3

Exogenous Calcium concentration can influence growth and morphogenesis in the hyphae of *Basidiobolus ranarum*

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Calcium ions have been implicated to regulate numerous activities in eukaryotic cells including cell polarity. We have evaluated the effect of extracellular calcium on tip growth, branching and nuclear positioning in *Basidiobolus ranarum*. Tip-growth of the hyphae occured at a slower rate when exogenous calcium was absent (0 mM Ca^{2+}). At higher Ca^{2+} concentrations (1 mM Ca^{2+} & 10 mM Ca^{2+}), hyphae grew faster. The slow growing cells also appeared shorter in length and smaller in volume but usually larger in diameter compared to the rapidly growing cells. Tip-branching was almost exclusively observed at low calcium but side branching was exhibited at both low and higher calcium. Whereas the nuclei at low calcium appeared to be round, the nuclei at higher calcium were often oval in shape. The average nuclear position relative to cell length remained constant irrespective of the concentration of exogenous calcium. Calcium ions may be influencing the growth and morphology of the hyphae by regulating localized cell wall deposition, polarized distribution of organelles and the cytoskeleton.

4

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The use of laser scanning confocal microscopy to characterize ectomyocrrhizas of *Pinus strobus* L. and to localize associated bacteria.

Laser scanning confocal microscopy (LSCM), light microscopy (LM) and epifluorescence microscopy (FM) were used to observe the extramatrical hyphae, mantle patterns and associated bacteria on mycorrhizal tips of *Pinus strobus* L. seedlings grown in pot cultures. *Laccaria* sp. and *Tuber* sp. formed ectomycorrhizas with *P. strobus*, while *Phialophora finlandia* Wang & Wilcox and E-strain (*sensu* Danielson 1982) formed ectendomycorrhizas. Distinct mantle patterns and cystidia were observed with greater resolution using LSCM, and intracellular hyphae were visualized in three dimensions. Trypan blue penetrated fresh whole mounts to 20 µm and was an excellent stain for visualizing fungal hyphae and bacteria with LSCM. Fluorescein isothiocyanate (FITC) and acridine orange were used in conjunction with LSCM and FM to localize bacteria on ectomycorrhizal tips. With LSCM, bacteria were visible in the surface mucigel, and optical sectioning through the root tip showed that bacteria were also present within the mantle. LSCM is a non-intrusive and fast method for visualizing mycorrhizal structures and their associated bacteria on fresh, whole root tips.

5

Genetic Mapping of Molecular Markers Linked to the *Avr1a* Avirulence Gene in the Soybean Pathogen *Phytophthora sojae*

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Avirulence against the *Rps1a* resistance gene, in a cross between two races of *P. sojae*, segregated as a single dominant gene, *Avr1a*. Random amplified polymorphic DNA (RAPD) analysis identified 192 primers that can distinguish between the two races. Bulked segregant analysis identified six primers, amplifying 4 dominant and 2 co-dominant markers, that can distinguish between bulks of DNA derived from avirulent and virulent F_2 progeny. The closest marker is 6.5 cM from the *Avr1a* locus. Four RAPD markers were used as probes for Southern blot analysis of digested parental DNA, and were converted to co-dominant RFLP markers. This revealed that the RAPD markers represented single to low-copy number sequences in the *P. sojae* genome and will, therefore, be important in a map-based cloning approach to isolating *Avr1a*. The RFLP markers displayed skewed segregation ratios in the F_2 population favouring homozygous genotypes, particularly for markers close to the *Avr1a* locus. This could indicate a lack of chromosome co-linearity, high frequency gene conversion, or non-reciprocal crossing over in the *Avr1a* region.

6

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Energy Dispersive X-ray Analysis of White Spruce Seeds and Somatic Embryos

Somatic embryos are produced in tissue culture and morphologically resemble natural zygotic embryos. Using explant tissue (usually seed tissue) embryogenic callus is produced, which further develops hundreds of somatic embryos. Mass propagation of embryos holds great potential for reforestation applications. Somatic embryos of white spruce (Picea glauca) were obtained from Dr. L.C. Fowke from the University of Saskatchewan. EDX analysis was performed on globoids (phytate storage in seed protein bodies) and Fe-rich particles (possible Fe-associated phytate deposits in plastids destined to become chloroplasts). Elemental compositions of globoids and Fe-rich particles in various regions of the female gametophyte, zygotic embryos and somatic embryos were very similar. Two noticeable exceptions were found. First, the globoids in the procambium of zygotic embryo cotyledons had significant Fe levels, which was not observed in somatic embryos. Secondly, Fe-rich particles generally had lower levels of Fe in somatic embryos. The production of somatic embryos similar to zygotic embryos is useful for biochemical and physiological studies of embryo development. However, since somatic embryos lack the highly nutritional female gametophyte they are potentially starting with considerably less nutrients than zygotic embryos. Currently tests are being performed to try and increase the levels of P in somatic embryos.

7

The effects of UV-A irradiation on the production of phenolic compounds in seedlings of *Acer saccharum* and *Acer platanoides*

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One-month-old seedlings of *Acer platanoides* and *Acer saccharum* each with one pair of leaves were determined to react differently in response to UV-A radiation. At the onset of the experiment the red-coloured *A. platanoides* seedlings contained higher concentrations of anthocyanins and other flavonoids in comparison to the green-coloured *A. saccharum* seedlings, both species growing outdoors. The seedlings of both species were treated for five days with 366 nm radiation and fertilized with a mixture of 200 ppm copper and 200 ppm nickel plus 20 ppm zinc as their sulphates. The roots and shoots of the two species reacted differently to UV stress. The roots always demonstrated an increased concentration of compounds absorbing 525, 325, and 280 nm radiation yet the shoots synthesized less of the compounds absorbing at 280 nm. The roots consistently synthesized more of the UV-absorbing compounds in response to the treatment compared to the shoots, which were irradiated directly. Due to the changing light conditions and increased pollution on earth it is necessary to determine the mechanisms utilized by plants in response to stress conditions (Day *et al.* 1992). Therefore, further investigation is needed into the mechanisms of information transfer and on the identification of compounds which differ in the shoot and root after treatment, with emphasis on those compounds absorbing 280 nm radiation.

8

The UVA induced resistance to UVB stress in relation to photosynthetic carbon metabolism in *Brassica napus* cv Topas.

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Brassica napus cv Topas plants grown under visible (400-700 nm) plus UVA (320-400 nm) light are better able to withstand anatomical and biochemical damage caused by UVB (280-320 nm) exposure than plants grown under visible light alone. Plants grown under visible plus UVA light assimilate CO_2 at the same rate as plants grown under visible light but show a decreased capacity for starch and sucrose biosynthesis. These plants may be partitioning their carbon to another pathway. We hypothesized that carbon is shunted to amino acid biosynthesis. The pool sizes of free amino acids in leaves of plants grown under [i] visible, [ii] visible + UVB, [iii] visible + UVA and [iv] visible + UVA + UVB, light regimes were measured to test this hypothesis.

9

Ruchira M. Sud and Nancy G. Dengler. Department of Botany, University of Toronto, Toronto. **Distribution of chlorophyll and photosynthetic enzymes in the variegated C4 grass, Stenotaphrum secundatum.**

Leaves of variegated St. Augustine grass, Stenotaphrum secundatum, a C4-NADP-ME species, have longitudinal stripes of white tissue, caused by "invasions" of genetically white layers into green chlorophyll-containing layers. In this study, we analyzed the pattern of white sectors and chlorophyll distribution using bright field and fluorescence microscopy of fresh sections. Using the same leaves, we examined the distribution of the photosynthetic enzymes, ribulose 1,5 bisphosphate carboxylase (RuBPCase) and phosphoenolpyruvate carboxylase (PEPCase) using immunolocalization of embedded tissue. We found that RuBPCase accumulated in bundle sheath tissue of the green stripes, but that PEPCase accumulated in a mesophyll-specific manner in both green and white tissues. These observations indicate that chlorophyll synthesis and RuBPCase expression share the same regulatory pathway, but that expression of PEPCase is independent.

10

Influence of ions on secondary metabolites in plant shoots

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High concentrations of metals in soils has been a growing concern, especially in areas which are heavily populated by vegetation. If a plant uptakes a toxic concentration of a metal, and we eat the plant, will it be toxic to us? A lack of selenium, on the other hand, can cause diseases in humans. This study found that individual solutions of nickel and copper, in 200 ppm concentrations, and cadmium and cobalt, in 20 ppm concentrations, were all readily uptaken by *Tradescantia* plants. The metals and concentrations represent those which are typically found in soil near Sudbury, Ontario. Ni, Cd, Cu, and Co were all uptaken by the plants and were transported to the top of the shoots in various proportions. The metals caused shrinkage of the tissues in the plant shoots. An imbalance in ions can cause changes in the biosynthesis of secondary metabolites, for instance coumarins and epicatechins. What effect does the concentration of metal ions have on the chelating process? Are complexes of ions with phenolic compounds located in specific tissues of a plant and in specific compartments of a cell? How can we explain that antimitotic effects of such plant extracts on animal cells but not on the promeristem of a plant?

11

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Phytate in stored barley grains and bean seeds.

Phytate is an important component of all seeds but the stability of phytate during long-term storage has not been defined. We have studied phytate in barley kernels (Hordeum vulgare L.) stored for up to 10 years and in bean seeds (Phaseolus vulgaris L.) stored for 14 months. We have also exposed the seeds to accelerated aging conditions of 41°C with and without elevated humidity of 75%. Under normal storage the barley kernels of two cultivars showed no decrease in phytate while two other cultivars lost 12 to 18% of their phytate. With storage at 41°C for 3 months in the dry the loss in phytate was less than 2% and there was a slight loss in germination. At 41°C and 75% RH the loss in phytate varied from 5 to 10% and no kernels germinated. The phytate in bean seeds did not change in 14 months of normal storage. With storage for at 41°C there was a 23% drop in phytate and at 41°C and 75% RH phytate levels fell by 27%. In both cases the aged beans lost all ability to germinate. Thus, under normal storage the phytate in both barley and beans was relatively stable. With accelerated aging the phytate within the beans was less stable than that within barley but barley cultivars varied in their response to both long-term normal and accelerated aging.

12

Genes encoded on a cyanobacterial plasmid are transcriptionally regulated by sulfur-availability and CysR.

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A cyanobacterial sulfur-regulated gene (*cysR*), which exhibits similarity to the Crp family of prokaryotic regulatory proteins, has recently been isolated and characterized. PAGE analysis of periplasmic protein extracts reveals that a *cysR*⁻ mutant fails to synthesize a 36 kDa polypeptide that is normally induced in wild-type cells that have been grown under sulfur-deficient conditions. Amino-terminal sequence of this protein was obtained and a synthetic oligonucleotide was used to isolate a clone containing a 1.9 kb *NruI/KpnI* fragment from a *Synechococcus* sp. PCC 7942 genomic library. RNA blot analysis indicates that this fragment encodes a transcript that is detectable in wild-type, but not *cysR*⁻ mutant cells that have been starved for sulfur. DNA blot analysis revealed that the 1.9 kb *NruI/KpnI* fragment is contained within the Ba4 *Bam*HI fragment of the endogenous 50 kb plasmid pANL. RNA blot studies indicate that the accumulation of a large number of pANL transcripts is regulated by sulfur levels and CysR. To date, we have identified 15 ORFs encoded on pANL which display significant similarity to proteins in the sequence database. Our work is currently focused on the function of pANL in four adaptive processes; (i) the role of five genes, including a chromate resistance determinant, a cysteine biosynthesis enzyme and an enzyme involved in glutathione metabolism, in the control of chromate levels within sulfur-deficient cells, (ii) the role of two genes, capable of complementing *E. coli cysKcysM* and *cysE* cysteine auxotrophs, in the biosynthesis of L-cysteine, (iii) the role of four genes in the transport of alternate sulfur-containing compounds such as thiocyanate and (iv) the role of three genes, which display similarity to a family of response regulators and sensors involved in mediating signal transduction, in cell sensing and the regulation of gene expression. The progress we have made in determining the function and biological significance of various pANL-encoded genes will be discussed.

13

The MADS-Box Protein AGL2 Is Able to Interact with Floral Regulators of *Arabidopsis thaliana*

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AGL2 (**AG**AMOUS-LIKE) is a member of a large family of putative transcription factors required for the regulation of floral morphogenesis in *Arabidopsis thaliana*. Sequence analysis reveals that these proteins share several common features such as the MADS-box which is believed to mediate DNA-binding, and the K-box, a putative protein-protein interaction site.

Among MADS-box proteins (to date, 24 distinct AGL sequences have been identified in *Arabidopsis*), AGL2 is most similar to AGL4 (93% identical amino acids). Commonly such a sequence similarity is interpreted as functional similarity. However, we have found experimental evidence that these two proteins are functionally diverse: AGL2 but not AGL4 is able to interact with AG in a two-hybrid approach and furthermore, AGL2 but not AGL4 can activate transcription in yeast. To gain a better understanding of AGL2's role during floral development, we have used a *GAL4*-based two-hybrid approach to screen an *Arabidopsis* cDNA expression library for proteins for AGL2 interactors. A truncated form of AGL2, AGL2-Δ2, which is unable to activate transcription on its own, was used to screen 1.8×10^7 transformants and 136 cDNAs were identified, representing at least 15 classes of AGL2 interacting-proteins. Among these are several MADS-box proteins with known floral specific expression patterns as well as several non-MADS proteins. Characterization of cDNAs encoding potential AGL2 interactors will be presented.

14

THE USE OF IMMUNO-GOLD CYTOCHEMISTRY TO DETERMINE THE SUB-CELLULAR LOCALIZATION OF THE 18-kDa HEAT-SHOCK PROTEINS IN MAIZE RADICLES.

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Heat-shock proteins (hsps), induced by temperature shifts and other stresses, are among the most highly conserved proteins. In plant species, the low-molecular weight heat-shock proteins (LMW hsps) are most abundant and conserved, in contrast to mammals in which the LMW hsps make up only a small fraction of total hsp content.

Immuno-gold cytochemistry and transmission electron microscopy were used to examine the distribution, subcellular and cellular localization of the 18-kDa hsp in Oh43 maize inbred heat-shocked and non-heat-shocked radicles. A recombinant protein, from our UWO 10 cDNA clone, produced in the pTrcHisB expression vector, was used to raise polyclonal antibodies. These antibodies, specific to the 18-kDa hsp proteins, were used to probe ultra-thin silver-gold sections of radicles, embedded in Epon-Araldite. Colloidal gold particles (15 nm) conjugated to goat-anti-rabbit IgG secondary antibodies were employed to detect the presence of antibody-hsp-18 complexes.

Analyses of the data demonstrate 18-kDa hsp localization in the cytoplasm and nucleus, in meristematic regions of the radicle, as well as to the inter-cellular spaces between cortex cells, in both heat-shocked and non-heat-shocked tissue. An approximate 15-fold increase of gold particles was found in the heat-shocked tissue relative to the non-heat-shocked tissue. Localization of the 18-kDa hsp, in heat-shocked roots, to structures apparently involved in "autophagic digestion" was observed. These findings suggest the role of LMW hsps as 'chaperones', and may suggest a further role in the regulation of osmotic pressure under heat-shock conditions. (Supported by an NSERC Reserach Grant to D.B.W.)

15

Antisense RNA *in situ* Hybridization Reveals the Localization of mRNAs of 18 kDa Heat-shock Protein Genes in Metal-Ion Insulted Maize Radicles

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Plant cells respond to environmental thermal shift by increasing the synthesis of the transcription and the translation of heat-shock(HS) genes and down regulating most other genes. Certain heavy metals also induce a similar response. Using a DIG-labelled antisense RNA probe synthesized from the open reading frame(ORF) of an 18 kDa heat-shock gene, *in situ* hybridization was carried out in maize radicles treated with different thermal shift regimes from one of two preshift temperatures(22°C or 27°C). In addition, Dot-blot hybridization analysis of extracted RNA and *in situ* hybridization were carried out on radicles derived from a series of metal-ion insults(3h). Hybridization was observed only when the shift temperature was above 35°C from either preshift temperature. Hybridization was restricted to meristematic cells in the thermal shift experiments. Hybridization was found in radicles treated with zinc and cadmium, localized more to the epidermal regions than to other cell types. Our data suggest that 35°C is the thermal shift threshold temperature for the heat-shock response in maize radicles. Meristematic cells respond to heat-shock first; epidermal cells are the first to respond to metal-ion insults. This latter result suggests that solubility of the insult agent is an important factor in mediating the insult regime.(supported by a NSERC Research Grant to DBW)

16

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Expression of peanut peroxidase in tobacco.

In order to learn what the roles of the peroxidase glycans may be, it was decided to use site-directed mutagenesis of the asparagine binding sites on the cDNA for the glycans. The mutated cDNA will be expressed in tobacco in order to obtain faithful expression of the glycans. Isolation of the transgenic peroxidase is needed to examine the glycan composition. Therefore, the intend is to use a His-tag on the cDNA and ultimately isolate the protein by nickel affinity. Some observations are shown as to plant growth and verification of the expression.

17

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GLYCAN ANALYSIS OF CATIONIC PEANUT PEROXIDASE.

Cationic peanut peroxidase has three N-linked glycan binding sites. Two forms, discriminated by mass and Con-A binding properties, are known to occur. The glycans have been purified and identified by trypsin digestion, HPLC separation and pronase digestion. Qualitative and quantitative analysis on the sugar composition of the glycans was carried out by HPLC. NMR analysis of the glycan structure is planned.

Once the structure of the glycans are known as well as the protein, studies on the role of the glycosidases will follow. We have observed microheterogeneity of the peroxidase glycans. How are these glycosidases influencing the stability, activity and perhaps transport of the peroxidase. Could such influence the function of peroxidase ?

18

Genome organization in *Zea mays* L.

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An increasing body of evidence indicates that the nuclei of eukaryotes are highly ordered, and that the arrangement of chromatin is important for complex biological processes such as transcription, RNA processing and DNA synthesis. Specific cell-type arrangements of chromatin have been observed in a variety of tissues from several plant and animal species. It seems likely that an ordered nucleus is a characteristic common to many species. The numerous aneuploids and euploid varieties of maize provide a unique opportunity to study the arrangement of chromatin in a nucleus. C-banding and *in situ* hybridization with DIG labeled probes have been employed successfully to determine the identity of chromosomes (1 to 10 and the B), the origin of a chromosome (maternal or paternal), and the location of sequences at different cell stages. These methods will be used to study the distribution of a 180 bp repeat that occurs at one to twenty three characteristic locations depending on the genetic background of a plant. The presence of arrays of this sequence result in thickened regions on the pachytene chromosomes referred to as knobs. Root -tip and tapetal cell nuclei from hybrid varieties of maize that were derived from parental stocks that differ in the amount of repeat sequences that are present in their genomes will be examined. Preliminary results will be presented.

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NOTES